Supplemental Methods:

The central nervous system, consisting of the cerebropleural ganglia, pedal ganglia, optic ganglia, the eyes and the statocysts, was dissected from thirty-one *Hermissenda crassicornis* specimens, weighing between 1g and 3.6g (Monterey Bay Abalone Co.). The tissue was immediately flash-frozen in liquid nitrogen for storage at -80°C. RNA was extracted with the RNeasy Universal Plus Midi-Kit (Qiagen). RNA concentration and quality were determined by Nanodrop and Bioanalyzer 2100 (Agilent). Beckman Genomics isolated mRNA and reverse-transcribed to cDNA, which was then PCR-amplified and barcoded for sequencing. Paired-end sequences were generated by an Illumina HiSeq 2500 high-throughput sequencer.

Raw Illumina transcript reads were analyzed by FastQC (Barbraham Bioinformatics) for transcript quality, and then trimmed to remove low quality reads using Sickle (https://github.com/najoshi/sickle). The remaining reads were then *de novo* assembled by Trinity software (Haas et al., 2013) (version r20140717) on the VELA high performance computer system at Georgia State University, which consists of four IBM System x3850 X5 servers running Linux. Trinity assembly was run using FASTQ sequence type, with 140Gb Jellyfish memory and 40 processors. Assembly statistics were generated using TrinityStats.pl. RSEM (Li, 2011) was used to evaluate transcript expression levels. TransDecoder (Haas et al., 2013) was used to identify candidate predicted protein coding regions within the transcripts, specifying a minimum length of 99 amino acids. A small amount of cross-contamination of cDNA from molluscan, plant, and insect species occurred during Beckman sequencing. Transcripts identified as matching a non-*Hermissenda* mollusc species were removed after transcriptome assembly, using a previously published filtering method (Senatore et al., 2015). Plant and insect transcripts were not removed, but are believed to represent a very small amount of transcripts.

BLAST+ version 2.2.29 (Camacho et al., 2009) was used either as locally installed software or on VELA. Nucleotide databases were generated from the published *Lymnaea stagnalis* transcriptome (Sadamoto et al., 2012) and *Aplysia californica* NCBI mRNA collection (Taxid:6500) on February 22nd, 2014, whereas the *Tritonia diomedea* transcriptome shotgun assembly database (Senatore et al., 2015) was generated by our lab. Protein databases were generated from SwissProt on May 24th, 2014 and from RefSeq on July 14th, 2014. These databases were searched by querying both the transcriptome and its TransDecoder-predicted proteins and then analyzing the results with Microsoft SQL. Functional annotations of gene ontology (GO) terms (Ashburner et al., 2000) were done using BLAST2GO (http://www.blast2go.com/b2ghome). KEGG (Kanehisa & Goto, 2000) pathways containing enzymes involved in learning or memory processes were then identified using the BLAST2GO interface.

Individual genes coding for proteins whose function is related to learning or memory were identified in the *Hermissenda* transcriptome by BLAST search using single gene queries from molluscan SwissProt or NCBI published genes. *Hermissenda* orthologue identity was confirmed by BLAST against NCBI or UniProtKB/SwissProt databases. Select identified gene sequences were translated to predicted amino acid open reading frames and aligned using ClustalW (Larkin et al., 2007). For membrane bound proteins, predicted transmembrane domain regions were identified using Phobius (Käll et al., 2004). For *Hermissenda* and *Tritonia* genes used in phylogenetic tree generation, serotonin (5-HT) receptor sequence (see below) predicted proteins generated from whole brain or whole body cDNA cloning and sequencing were used. *Hermissenda* and *Tritonia* dopamine receptor protein sequences were predicted from transcriptome sequences. A 15 amino acid-long predicted protein was deleted from the *Aplysia* dopamine receptor D2 protein sequence during alignment. GABA-B receptor and adenylyl cyclase (AC) predicted proteins were generated directly from transcriptome sequences. Unrooted phylogenetic trees were created by Maximum Likelihood with Jones-Taylor-Thornton model using MEGA6.06 software (Tamura K, 2011). Branch supports were provided using 500 bootstrap replicates. Gene identification numbers for all published genes are listed in Supplemental Table S2.

To clone 5-HT receptor genes, primers were designed against transcriptome 5-HT receptor sequences. Primers for 5-HT receptors are listed in Supplemental Table 3. Whole brain RNA was extracted as described above. cDNA was reverse-transcribed using SuperScript IV (Invitrogen). PCR amplification of 5-HT receptor genes was performed using *Taq* DNA polymerase, buffer, dNTP and magnesium chloride from ThermoFisher. DNA bands were excised using a Zymoclean Gel DNA Recovery Kit (Zymoresearch), ligated using pGEMT Easy (Promega), and transformed into JM109 competent cells (Promega). Resulting colonies were isolated and plasmids were extracted using a GenElute plasmid mini-prep kit (Sigma). Plasmids with inserts were sequenced by a **3730xl DNA Analyzer** (Life Technologies). Resulting sequences were verified against NCBI and UniProtKB/SwissProt databases. Three or more sequences resulting from separate PCRs, as well as the transcriptome sequence, were aligned using ClustalW to determine the most likely sequence for each gene.

Plasmids from *Hermissenda* 5-HT2a, as well as 5-HT transporter and pedal peptide 3 precursor, were *in vitro* transcribed to make synthetic RNA using the Ambion MegaScript RNA synthesis kit (ThermoFisher). Copy number was determined using resulting synthetic RNA Nanodrop concentration and sequence information for that gene. RNA was serially diluted to volumes calculated to contain standard amounts of each RNA strand, and reverse-transcribed using SuperScript IV (Invitrogen). Absolute qPCR was performed on an Applied Biosystems 7500 Fast Real-Time PCR system, using Perfecta SYBR green with low rox (Quanta Biosciences). Whole brain cDNA was compared in triplicate against the RNA standards. No-reverse transcriptase controls and no-template controls were subtracted from resulting values. To ensure that only a single amplification product was quantified, melt curves were determined for each qPCR trial, and resulting PCR products were run on a 1% agarose gel (not shown).

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